Recombinant Poxvirus comprising at least two cowpox ATI promoters

The invention concerns recombinant poxviruses comprising in the viral genome at least two expression cassettes, each comprising the cowpox ATI promoter or a derivative thereof and a coding sequence, wherein the expression of the coding sequence is regulated by said promoter or derivative thereof. The virus may be useful as a vaccine or as part of a pharmaceutical composition.

Background of the invention

Recombinant poxviruses are widely used to express foreign antigens in infected cells. Moreover, recombinant poxviruses are currently tested as very promising vaccines to induce an immune response against foreign antigens expressed from the poxvirus vector. Most popular are avipoxviruses on the one side and vaccinia viruses on the other side. US 5,736,368 and US 6,051,410 disclose recombinant vaccinia virus strain Wyeth that expresses HIV antigens and proteins. US 5,747,324 discloses a recombinant Vaccinia virus strain NYCBH expressing lentivirus genes. EP 0 243 029 discloses a recombinant vaccinia virus strain Western Reserve expressing human retrovirus genes. Fowlpoxviruses containing HIV genes in the viral genome are disclosed in US 5,736,368 and US 6,051,410.

To induce an effective immune response it is desirable to express not only a single protein of an agent against which an immune response is to be induced. Instead, it is preferred to express as many different proteins and epitopes of said agent as possible to obtain a broad and effective immunity against said agent. Thus, it might be advantageous to insert several different expression cassettes into the same poxviral genome if it is intended to use a poxvirus as a vector for vaccination. US 5,736,368 describes the construction of a recombinant poxvirus harboring expression cassettes for

the HIV-1 env gene and the HIV-1 gag-pol gene. For the expression of the proteins encoded by the different expression cassettes different promoters were used, namely the vaccinia virus D1 promoter and the 40K promoter. The disadvantage of this strategy is that the activities of the different promoters are not identical resulting in a different level of the proteins expressed from the different expression cassettes.

An almost identical expression level could be obtained if the promoters in the different expression cassettes in the poxvirus genome were identical. However, the disadvantage of this strategy is that there is a risk that undesired recombination events may occur between the homologous/identical promoter sequences. Indeed, it has been shown by Howley et al. (Gene (1996) 172, 233-237) that a recombinant vaccinia virus may be generated that comprises three p7.5 promoters in different locations of the viral genome; however, recombination occurred between the homologous promoter sequences resulting in a mixed genomic population of the recombinant poxvirus. Such a mixed and undefined genomic population that reflects the instability of the viral genome is not acceptable if it is intended to use a recombinant poxvirus for vaccination, in particular for the vaccination of humans.

Claims:

1. Recombinant poxvirus comprising in the viral genome at least two expression cassettes, each comprising the cowpox ATI promoter or a derivative thereof and a coding sequence, wherein the expression of the coding sequence is regulated by said promoter or the derivative thereof.

2. Recombinant poxvirus according to claim 1, wherein at least two expression cassettes are inserted into the same insertion site in the poxvirus genome.

3. Recombinant poxvirus according to anyone of claims 1 to 2, wherein the ATI promoter in at least one of the expression cassettes has the sequence of SEQ ID: No. 1

4. Recombinant poxvirus according to anyone of claims 1 to 2, wherein the ATI promoter in at least one of the expression cassettes is a derivative of the ATI promoter selected from

(i) subsequences of the sequence according to SEQ ID: No. 1

(ii) sequences having one or more nucleotide substitutions, deletions and/or insertions with respect to the sequence according to SEQ ID: No. 1 or with respect to a subsequence thereof,

wherein said subsequences and sequences are still active as promoter in the poxvirus.

5. Recombinant poxvirus according to anyone of claims 1 to 4, wherein the poxvirus is selected from the group consisting of orthopoxviruses and avipoxviruses.

6. Recombinant poxvirus according to claim 5, wherein the orthopoxvirus is a vaccinia virus and wherein the avipoxvirus is selected from canarypoxvirus and fowlpoxvirus.

7. Recombinant poxvirus according to claim 6, wherein the vaccinia virus is modified vaccinia virus strain Ankara (MVA), in particular MVA-BN and MVA 575, deposited under numbers V00083008 and V00120707, respectively, at the European Collection of Animal Cell Cultures (ECACC).

8. Recombinant poxvirus according to claim 7, wherein at least one of the expression cassettes is inserted in a naturally occurring deletion site of the MVA genome with respect to the genome of the vaccinia virus strain Copenhagen.

9. Recombinant poxvirus according to anyone of claims 1 to 8, wherein at least one of the expression cassettes is inserted in an intergenic region of the poxvirus genome.

10. Recombinant poxvirus according to anyone of claims 1 to 9, wherein at least one of the coding sequences codes for least one antigen, antigenic epitope, and/or a therapeutic compound.

11. Recombinant poxvirus according to anyone of claims 1 to 10 as vaccine or medicament.

12. Vaccine or pharmaceutical composition comprising a recombinant poxvirus according to anyone of claims 1 to 10.

13. Use of the recombinant poxvirus according to anyone of claims 1 to 10 for the preparation of a vaccine or medicament.

14. Method for introducing coding sequences into target cells comprising the infection of the target cells with the virus according to anyone of claims 1 to 10.

15. Method for producing a peptide, protein and/or virus comprising

a) infection of a host cell with the recombinant poxvirus according to anyone of claims 1 to 10,

b) cultivation of the infected host cell under suitable conditions, and

c) isolation and/or enrichment of the peptide and/or protein and/or viruses produced by said host cell.

16. Method for affecting, preferably inducing an immunological response in a living animal body including a human comprising administering the virus according to anyone of the claims 1 to 10 or the composition or vaccine according to claim 12 to the animal or human to be treated.

17. Method according to claim 15 comprising the administration of at least 10^2 $TCID_{50}$ (tissue culture infectious dose) of the virus.

18. A cell containing the virus according to any of claims 1 to 10.

19. A method for the production of a recombinant virus according to anyone of claims 1 to 10 comprising the step of inserting at least two expression cassettes into the genome of a poxvirus.